GB3 and Ubiquitin, when bound to AuNPs. We find no significant changes in slow HDX rates (5-300 min), suggesting that AuNP-induced structural changes are small for these two proteins. Together, these results support a model where most of a protein's native contacts are preserved upon adsorption, although larger changes may occur over long timescales.

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3D Reconstruction of the S885A Mutant of the Human Mitochondrial Lon Protease

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The Lon protein is a protease belonging to the superfamily of ATPases Associated with diverse cellular Activities (AAA+). Its main function is the control of protein quality and the maintenance of proteostasis by degrading misfolded and damaged proteins, which occur in response to numerous stress conditions. Lon protease has been also shown to participate in regulation of levels of transcription factors that control pathogenesis, development and stress response. Furthermore, it seems to play an important role in aging, and it is supposed to be involved in mtDNA replication, translation, or repair. We focus our interest on the structure of human mitochondrial Lon (hLon) protease whose altered expression levels are linked to some severe diseases, such as epilepsy, myopathy, or lateral sclerosis.

At the moment, it is assumed that Lon subunits assemble into oligomeric structures whose conformations are supposed to differ at ATP, ADP, and protein substrate binding. However, neither the full 3D structure of the Lon holoenzyme nor the mechanism of Lon's action is known. Several sub-structures of bacterial and human Lon have been resolved by X-ray scattering, and one 3D structure of an E. Coli Lon dodecamer active at physiological protein concentrations was resolved with electron microscopy.

Here, we present two conformations of an ADP-bound hLon S885A mutant obtained as a result of cryo-EM data analysis. The S885A mutant has a point mutation on the proteolytic domain, which completely disables its proteolytic function but does not affect its ATP-binding properties. The 3D reconstructions reveal that human Lon is a hexamer whose proteolytic and ATPase domains are arranged into a helix. The opening and pitch of the helix depend on the N-terminal domain interactions. These structures provide an insight toward the understanding of the protein mechanism of action.

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Structure and Dynamics of the EIIC Sugar Uptake System

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The phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) is a sugar uptake system unique to bacteria. It is a multicomponent system consisting of several cytosolic proteins and a dimeric transmembrane protein (EIIC in most PTS systems), which transports extracellular sugar across the membrane. Although EIIC is a uniporter, it is able to drive concentrative transport of its ligand because the sugar is phosphorylated by a cytosolic protein, EIIB, while still bound to the transporter. Phosphorylation prevents the sugar from escaping the cell and primes it for consumption by the cell. Little is known regarding the mechanism of sugar translocation and phosphorylation. Currently, the only available crystal structure of an EIIC is that of the N,N′-diacetylchitobiose transporter, bcChbC (1). We have proposed a mechanism for sugar translocation and phosphorylation, and we will report our progress in characterizing the mechanism.

Reference

1. Cao Y, Jin X, Levin EJ, et al. Crystal structure of a phosphorylation-coupled saccharide transporter. Nature. 2011;473(7345):50-4.

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Predicting the Effects of Clinically Observed Kinase Mutations using Molecular Modeling and Machine Learning Algorithms

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Many cellular processes are impacted by signaling through receptor and non-receptor kinase proteins. These include such diverse cellular actions as proliferation, differentiation, and motility, as well as tissue level phenomena such as angiogenesis and development. This important role in the cell is reflected also in the relative overrepresentation of kinases among known cancer mutations to proteins. In order to better understand the functional effects of these mutations, we have developed computational methods that seek to predict the effect of point mutations on kinase activation. By predicting whether a given mutation causes a kinase to be more active, we can gain insight into the overall impact of the mutation on cell phenotype and give insight to clinicians on patient cohorting for efficacious treatment with targeted kinase inhibitors. We have developed two separate but complementary methods to predict kinase activation status. The first uses molecular dynamics (MD) simulations and scoring criteria to predict if a mutation preferentially stabilizes the protein's active state. As a complimentary approach to MD, we have developed machine learning techniques that utilize the method known as support vector machines to predict whether mutations in a large number of kinases (>450) are activating. This method has proven to be almost as effective at predicting activation mutations as the mechanistic picture gained from MD simulations. We think these methods are both broadly applicable and have the potential to greatly impact both our understanding of mechanisms of kinase activation as well as to guide best practices in the clinical setting of targeted therapy in cancer treatment.

1889-Pos Board B26

Activation Mechanism of a Signaling Protein at Atomic Resolution

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The interconversion between the inactive and active state is the heart of signaling. This process has traditionally been described by the two corresponding structures, sometimes complemented with kinetic data. However the question of how these folded states interconvert is largely unknown due to the inability to experimentally observe the transition pathways.

Here we present a recent investigation of the full free energy landscape of the receiver domain of the response regulator NtrC (NtrC) by combining several computational methods including the string method, Markov state models of massive unbiased MD simulations, and long MD simulations on ANTON, with new NMR structural data.

The results unveil several unexpected features underlying efficient signaling: The active and inactive states have to be considered purely in kinetic terms. The functional need of attaining a stable and well-defined conformer, crucial to the active form of the protein, is absent in the inactive state. The inactive state comprises a structurally heterogeneous collection of sub-states that interconvert on timescales shorter than the transition to the active state. The transitions between the two functional states occur through multiple pathways characterized by transition states with dramatically different structural features. In addition to this entropic lowering of the transition barrier, a number of polar side-chains engage in unspecific transient interactions during the barrier crossing and thus make the activation mechanism flexible, efficient and robust. These novel findings challenge the structural paradigm of signaling and may represent general features for functional conformational transitions within the folded state.

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Crystal Structures of Trehalose Synthase from Deinococcus Radiodurans Reveal a Closed Conformation for Intramolecular Isomerization Catalysis and Mutant Induction of an Active-Site Aperture

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Trehalose has been used in food, cosmetic, and biotechnological industries due to its exceptional stability. Trehalose synthase (TS) catalyzes a simple conversion of inexpensive maltose into trehalose and hence has a great potential. TS consists of a catalytic (β/α)8 barrel, a subdomain B, a C-terminal β domain and two TS-unique subdomains (S7 and S8). The apo TS structures from Mycobacterium smegmatis and M. tuberculosis showed an unusual inactive conformation, in which the S7 loop blocks the substrate-binding pocket. Here we report structural and mutational studies of TS from Deinococcus radioduran (DrTS). The complex structures of DrTS with the inhibitor Tris share high homology with the substrate-bound sucrose hydrolase, amylosucrase, and sucrose isomerase, particularly virtually identical active-site architectures. A maltose was modelled into the active site and subsequent mutational analysis suggested that Tyr213, Glu320, and Glu324 are essential for the TS activity. In addition, the interaction networks between subdomains B and S7 seal the active-site entrance. Disruption of such networks through replacement of Arg148 and Asn253 with alanine resulted in a decreased isomerase activity but an increased hydrolase activity. The R148A and N253A structures showed a small pore created for water entry.